(overlapping around 67 ppm in F2 and 130 ppm in F1) correspond to β-sheet like conformations.

2349-Pos Board B41
The Function of Amot Dimerization in Lipid Binding
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Amots are adaptor proteins which coordinate signaling for cellular differentiation and proliferation. Their ACCH domain binds lipids with specificity leading to membrane deformation. A critical feature of Amot proteins is a novel lipid-binding domain, the Amot coiled-coil homology (ACCH) domain, which has the ability to selectively bind monophosphorylated phosphatidylinositols (PI) as well as target transcription factors to the nucleus. Understanding the biophysical mechanisms involved in lipid binding may provide pathways to modulate protein sorting and downstream signaling events inducing cellular differentiation, cancer cell proliferation, and migration. The central hypothesis is that characterizing Amot lipid-binding events will enable specific modulation of Amot isozyme for the prevention of ductal cell hyperplasia progression into breast cancer tumors. Synthetic membranes are used to demonstrate the role of dimerization in the ability to maintain ACCH-lipid binding activity. The specific aims of this work are: 1) Delineate the properties of the ACCH domain that provide for a dimer switch; and 2). Define the lipid-interaction properties of the ACCH domain homo- and hetero-dimer.
Site-directed mutagenesis was employed to probe the specific contributions of 37 selected lysines and arginines. We first measured the mutation stability through DSF, and then for their ability to dimerize using DLS. We then compare that information with that garnered from intrinsic tyrosine absorbance to understand the changes in structural conformations as a function of the mutation and lipid binding. As a result, we look to provide further information about which conserved residues participate in dimerization as a mechanism to control hetero- and homo-dimer formation.
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2350-Pos Board B42
Membrane Permeabilization by Holin like Proteins CidA/LrgA
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Holins are a large family of membrane proteins that control the activity of bacteriophage-encoded murein hydrolases by regulating their access to the peptidoglycan substrate. The two S. aureus membrane proteins CidA and LrgA exhibit holin and, respectively, anti-holin like properties. They are believed to play an important role in the formation of biofilm by controling bacterial cell lysis during bacterial programmed cell death. This resembles the behavior of apoptotic (e.g. Bax/Bak) and antiapoptotic (e.g. Bcl-2/Bcl-XL) proteins in eukaryotes. Pure preparations of recombinant CidA/LrgA were functionally reconstituted into synthetic lipid vesicles, as confirmed by circular dichroism spectroscopy and transmission electron microscopy. Fluorescent markers were entrapped into the protein reconstituted vesicles. A new method was developed to study the protein-induced leakage from vesicles since the conventional leakage assay could not be applied to CidA/LrgA. Our initial study has revealed that CidA induces leakage of small dyes. Ongoing studies include leakage of larger molecules to determine the dimension of the pore formed by CidA and characterization of the role played by LrgA in pore formation. This research will provide insight into the in vivo function of Cid/Lrg proteins and shed light on bacterial holin-like proteins.

2351-Pos Board B43
Structural Characterization of A. Thaliana Heterotrimeric G-Proteins
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Heterotrimeric G-proteins, consisting of three distinct subunits Galpha, Gbeta, and Ggamma, constitute one of the most important signal transduction systems in eukaryotes. In mammals they function as molecular switches in responses to external stimuli such as light and drugs. The activation mechanism for the mammalian heterotrimer is well known while in plants it is just inferred by analogy with the mammalian complex. Plant heterotrimeric G-proteins have been shown to be involved in multiple phisiological process, like regulation during development of leaf shape, cell proliferation, lateral root formation, stomatal density as well as control of post germination process (1); despite this, the available set of subunits looks limited compared to their mammalian cognates. In the Arabidopsis genome only one gene is present for the alpha and beta subunit while at least 3 genes have been discovered to belong to gamma subunit providing functional selectivity and specificity to the heterotrimer (2). In order to elucidate the activation mechanism in plants and better understand the G-protein-mediated molecular pathways, a structural investigation of the A. thaliana complex has been undertaken . Heterotrimeric G-protein subunits (GPA1, AGB1, and AGG2) from A. thaliana were cloned and purified. GPA1 was purified from P. pastoris while AGB1 and AGG2 from E. coli. For all the three subunits SAXS data are avalaible while for AGG2 subunit several crystallization trials have been put in place. Coexpression of AGB1-AGG2 dimer is currently in progress in E. coli expression system. Data from X-ray diffraction and small angle X-ray scattering will be collected in order to be able to move to the next step: the heterotrimer full reconstitution.

2352-Pos Board B44
Conformational Dynamics During Spliceosome Assembly Investigated by Single-Pair FRET
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The ribonucleoprotein (RNP) machinery of the spliceosome is composed of several subunits, which assemble stepwise during the process of splicing. U2 auxiliary factor (U2AF), a heterodimer comprised of a large (65 kDa) and a small (35 kDa) subunit, is involved in the early recognition of the intron and stabilization during splicing reactions. U2AF65 specifically recognizes the polypyrimidine tract in pre-mRNA introns and additionally contacts further splicing factors, such as mBBP/SF1 and SAP155 [1,2]. U2AF65 contains three RNA recognition motifs (RRM), where RRM1 and RRM2 mediate RNA binding while RRM3 is a U2AF homology motif (UHM) that mediates the interaction with U2AF35 [3].
A nuclear magnetic resonance spectroscopy study of recombinant human U2AF65 (RRM1-RRM2) showed a closed conformational state for the splicing factor in absence of RNA, while an open conformation is induced upon binding to polypyrimidine stretches [4].
Here, conformational subpopulations of the protein were investigated using single-pair FRET in solution with multiparameter fluorescence detection and pulsed interleaved excitation [5]. Information on FRET efficiency, stoichiometry, and lifetime revealed different conformational states dependent on substrate recognition and provided clues for dynamic motions of the splicing factor. These were further analyzed on a total internal reflection microscope using molecules immobilized in lipid vesicles. Changes in FRET efficiency over time showed a highly flexible U2AF65 protein with stabilization of specific conformational states upon RNA binding. Single-pair FRET measurements provide detailed insights into the mechanistic action of polypyrimidine tract recognition.
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2353-Pos Board B45
Eisosomes and Plasma Membrane Domain Formation
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A vast body of evidence coming from different microscopy techniques has been instrumental in concluding the long debate on whether biological membranes presented lateral segregation of proteins and lipids. Currently, the existence of membrane domains in both eukaryotes and prokaryotes is common ground. However, the mechanisms that sustain membrane domain formation and maintenance remain largely unknown. Our work is focus on the study of eisosomes, recently discovered plasma membrane domains in S. cerevisiae. We showed that Pil1 and Lsp1, the major proteinaceous components of eisosomes, are able to form self-assemblies that bind and curve membranes both in vivo and in vitro. We also showed that Lsp1 and Pil1 membrane-sculpting abilities are associated with the generation and organization of membrane domains (1,2). Thus, our current work supports the hypothesis that a mechanism for membrane eisosome domain formation is membrane curvature generation directed by Pil1-Lsp1 assemblies. To address this hypothesis we are applying Fluorescence Correlation Spectroscopy and Number and Brightness Analysis to describe the dynamics of Pil1 and Lsp1 in vivo. We are studying the oligomeric state and concentration of these proteins in the cytoplasm soluble fraction and in the plasma membrane. Also we plan to study the kinetic parameters of Pil1, Lsp1 and plasma membrane interactions during eisosome biogenesis and also during stationary phase.